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November 21, 2000

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Environmental Assessment
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Telephone: 919-541-4847

RE: Protocol 1416-003 - Oral (Drinking Water) Developmental Toxicity Study of Ammonium Perchlorate in Rats

Dear Ms. Jarabek:

Noted below are responses to Dr. Kimmel's comments and questions of 18 October 2000 for the above-referenced study.

(1) Per previous emails, this is one "piece" of the overall effects protocol that needs to be finalized for the peer review. Can the appropriate appendices (e.g., D, Certificate of Analysis and H, Quality Assurance) be signed and the report issued separately? I think I got agreement from Mike G. on this last week?

The study protocol was amended to allow the final report to be issued as two stand-alone GLP compliant reports. One report presents the methodology and results from Parts A, B and C of the study. The other report, the developmental toxicity portion of the study, presents the methodology and results from Part D of the study. The report for the developmental toxicology portion of this protocol (1416-003D) was finalized on 4 August 2000. The QA statement (Appendix H) was signed by our QA on that date. The Certificate of Analysis (Appendix D) was obtained from Aldrich and is not a signed document.

Because this report is finalized, it would be necessary to amend the final report to include any of the following responses to your comments and questions, if you felt it were necessary to include any of these items in the report.

(2) Several issues (4) re: study design and animal husbandry require clarification from Argus:

Can we assume that the missing consumption data (1-2 points in many of the groups, Tables B1 & B2) did not affect the results? This was not noted in the report. Can the data be provided to us (a) now and (b) in the final report?

It was not possible to include feed consumption data for a few rats at several summarized intervals because of spilled feed or incorrectly or not recorded feed consumption values, as noted on the individual tables. It was not possible to include water consumption data for a few rats at several summarized intervals because of spilled water or incorrectly or not recorded water consumption values, as noted on the individual tables. These deviations did not affect the outcome or interpretation of the study because sufficient data were collected to evaluate these parameters.

The dosage concentrations for the week of 14 Feb 00 - 20 Feb 00 were "based on water consumption data from the previous week because the latter data were not available...." This week would have been the first week of gestation, since the "cohabitation period" was from 14 Feb - 23 Feb 00. The Argus report indicates "This deviation did not affect the outcome...." Gary is concerned that Argus can support this, especially since this is the period when preimplantation effects would be initiated.

The actual consumed dosages during this period (the beginning of gestation) calculated using the concentration of test substance offered and the actual waster consumption and body weights of the rats were at approximately the target dosage levels.

Breeder males were also exposed. Can we assume that the females were only bred with males exposed to the same exposure concentration? This is not stated anywhere.

Female rats in the 0.01 mg/kg/day exposure group that did not mate within the first five days of cohabitation were assigned alternate male rats that had mated female rats in the 0 (Carrier) or 0.01 mg/kg/day exposure groups.

Appendix E: Environmental and Husbandry Page 227 - There was a maximum temperature of 100.0 F. There were 8 points out-of-range. Page 228 indicates the 100.0 readings were false temperature recordings as indicated by facility deviation. It is not clear what is meant by this, whether this includes the three 84F readings, and how Argus can justify saying that these deviations did not adversely affect the outcome or interpretation of the study.

On 16 February 2000, the transformer for the Vivarium Temperature and Relative Humidity Monitoring System was disconnected in order to add additional equipment in the area. As a result, erroneous temperature readings of 100.0°F were recorded at 5 timepoints between 10:00 and 14:00 in study room 14. This deviation did not affect the outcome or interpretation of the study because the actual temperature in the area was below that required to set off a high temperature alarm (85°F).

(3) Maternal endpoint issues (2):

Three dams in the 30mg/kg/day group showed an increase in localized alopecia that was statistically significant. The report indicates the increase was not considered exposure-related, since "this observation is commonly observed in rats in the laboratory environment." This statement is not supported and makes little sense. None of the other groups showed any signs of alopecia, and Argus did not include this information for the historical controls; consequently, the "commonly observed" assertion is not supported. Moreover, if this is "commonly observed" to the extent that some importance cannot be assigned to a statistically significant finding in a study with less-than-optimal power, then the endpoint is of no value in the analysis. The alopecia was observed in the three females over 9-11 days during mid-late gestation; not just on a single day. This finding should be considered biologically significant and exposure-related.

The study was conducted in accordance with OPPTS 870.3700 and the appropriate number of rats were included (a sufficient number of animals to yield approximately 20 animals with implantations at necropsy), which should provide sufficient power. We have compiled data from the last 10 developmental toxicity studies conducted at Primedica Argus. The incidence of localized alopecia in this study is within the range observed historically at this Testing Facility. [In 249 control group rats from 10 developmental toxicity studies conducted at the Testing Facility from May 2000, to November 2000, the average number of rats with localized alopecia of the limbs was 1.5 (range of 0 to 4 rats out of 25)]. Not only is the incidence within the historical control range, there are no observations of alopecia in any of the other groups and therefore, no exposure-dependent response. In the professional judgement of the Study Director, this observation is not biologically significant, and does not warrant a change in the conclusions already reported.

On page 22, footnote "a" indicates that the number of corpora lutea in one animal was incorrectly recorded. However, Table B17 does not indicate this missing data. Argus needs to clarify this apparent inconsistency.

The number of implantations for rat 19139 were 7/10 and the corpora lutea were recorded as 8/9 (one corpora lutea on the right ovary was missed. The corpora lutea were tabulated as 8/10.

(4) Developmental toxicity endpoints (1 question):

The report indicates that there was evaluation of the cartilage. Argus did not use the double-staining technique that is commonly used for staining bone and cartilage, nor do they indicate what alternate technique was used. Moreover, neither the report nor the historical database (Appendix G) provides data on the cartilage assessment. This parameter would provide additional data that may be helpful in assessing the potential effect of the exposure on overall skeletal-cartilage development.

Although we did not double-stain the fetuses, our procedure for the examination of skeletal specimens as outlined in our facility SOP includes the examination of cartilage. The cartilage is very visible in any good skeletal preparation and an observation of absent indicates that both bone and cartilage are not present. This method was described in the study protocol, which the EPA reviewed before the start of the study. An abstract presented at the European Teratology Society Meeting in September of this year[a], contends that double staining is meaningless because currently there is no systematic method of evaluating the cartilage. They propose three methods: 1) assess and record the structure and stage of development of both cartilage and bone formation; 2) assess and record the structure and stage of development of bone formation, and record only changes to the structure of the cartilage; or 3) record only changes in cartilage formation associated with ossification changes. The last method is used at Argus and can be performed without double staining. Additionally, the double staining may eliminate some of the bone staining.

a. A Critical Assessment of Double Staining for Fetal Specimens as a Routine in Prenatal Development Studies, UK Industrial Reproductive Toxicology Discussion Group, Parkinson, M. and Bailey, G.

I hope these responses have adequately addressed your comments. If you have any further questions, please do not hesitate to contact me.

Sincerely,

Raymond G. York, Ph.D., DABT
Associate Director of Research and
Study Director

RGY:vas
cc: Joan Dollarhide